



PATENT

Our Docket: 66661-016 (P-IS 4367)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:) Confirmation No.: 3087
Biaoyang Lin) Examiner: M. Davis
Serial No.: 09/729,653) Group Art Unit: 1642
Filed: December 4, 2000)
For: PROSTATE-SPECIFIC POLYPEPTIDE))
PAMP AND ENCODING NUCLEIC)
ACID MOLECULES)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

22/c

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Sir:

I, Biaoyang Lin, Ph.D. declare as follows:

1) I am the Biaoyang Lin who is named as the inventor on the above-identified patent application.

2) I understand that the claims of the subject application stand rejected, in part, because it is alleged that the claimed PAMP polypeptides lack utility.

3) The above-identified patent application asserts that the claimed PAMP polypeptides can be used to generate antibodies. I, or those working under my direction and supervision, have performed experiments to confirm that PAMP polypeptides are useful for generating antibodies that detect PAMP polypeptides expressed in vivo.

EXHIBIT A

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4) A mouse monoclonal antibody, designated 9E1-N1, was generated against a PAMP polypeptide encoded by nucleotides 2590-2968 of SEQ ID NO: 1. Monoclonal antibody 9E1-N1 detected both a 151 kDa PAMP polypeptide in an LNCaP cell lysate and a 61 kDa PAMP polypeptide in serum samples from prostate cancer patients by Western blotting (Figure 1, lanes B and D, respectively). Specificity of 9E1-N1 for these PAMP polypeptides was confirmed using a competition assay in which the antibody was pre-incubated with the PAMP polypeptide prior to Western analysis (Figure 1, lines A and C, respectively). Therefore, we believe that the 61 kDa PAMP polypeptide contained in serum is a truncated form of the 151 kDa PAMP polypeptide expressed in LNCaP cells.

Monoclonal antibody 9E1-N1 also detected a 151 kDa PAMP polypeptide in human tissue samples, with high levels of the polypeptide observed in ovary and testis, low levels in prostate and placenta, and undetectable levels in heart, liver and pancreas (Figure 2).

5) Immunohistochemical staining with monoclonal antibody 9E1-N1 was used to determine PAMP polypeptide expression in normal and prostate cancer human tissue sections. As shown in Figure 3, high levels of PAMP polypeptide expression in prostate cancer specimens (panels A and B) were detected, as compared to low levels of PAMP polypeptide expression in normal prostate and adjacent tissues (panel C). These results corroborate the teaching in the specification that the claimed PAMP polypeptide is expressed in nature, and indicate that PAMP polypeptide expression is higher in cancerous prostate tissue than in normal tissue.

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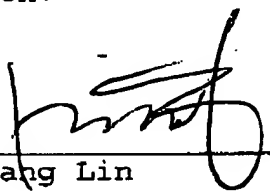
6) Monoclonal antibody 9E1-N1 further was used to distinguish between normal sera and sera obtained from prostate cancer patients. In this study, sera were obtained from 10 normal individuals, 10 early stage prostate cancer patients, and 10 advanced stage prostate cancer patients. Serum samples were diluted 1:10, and Western blot analyses were performed using both 9E1-N1 and an anti-prostate specific antigen (PSA) antibody. As illustrated in Figure 4, a Western blot showing two representative serum samples of each type, monoclonal antibody 9E1-N1 detected high levels of PAMP polypeptide expression in sera obtained from patients having advanced prostate cancer, lower levels of PAMP polypeptide expression in sera obtained from patients having early prostate cancer and undetectable levels of PAMP polypeptide expression in sera obtained from normal individuals. As summarized in Table 1, elevated levels of PAMP polypeptide expression were detected in 9 of 20 prostate cancer serum samples, while the PSA antibody was positive in 7 out of 20 prostate cancer serum samples. Moreover, monoclonal antibody 9E1-N1 appeared to have greater sensitivity than anti-PSA in detecting sera of early stage prostate cancer patients, as indicated by the ability of 9E1-N1 to detect four early stage prostate cancer serum samples while anti-PSA was unable to detect any (See Table 1).

7) These results corroborate that PAMP polypeptides have utility in generating antibodies useful for detecting PAMP polypeptides expressed *in vivo*. Moreover, these results indicate that PAMP polypeptide is differentially expressed in prostate

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cancer tissue and sera from prostate cancer patients, as compared to normal tissue and sera from normal individuals, and indicate that anti-PAMP antibodies are useful for prostate cancer diagnosis.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.


Biaoyang Lin

082603
Date

Table: Summary of western analysis

Samples	Sample ID	Gleason Scores	PAMP-9E1N1	PSA
Advanced CaP	12872M	3+4/10	61 kDa	Positive
Advanced CaP	14770C	3+3/10	negative	DID NOT DO
Advanced CaP	17330O	7/10	negative	Positive
Advanced CaP	19399M	4+5/10	61 kDa	Positive
Advanced CaP	19598G	4+3/10	61 kDa	Positive
Advanced CaP	20350W	n/a	negative	Positive
Advanced CaP	20773L	n/a	61 kDa	Positive
Advanced CaP	20937H	n/a	negative	negative
Advanced CaP	21433C	4+5/10	61 kDa	negative
Advanced CaP	21729M	9/10	negative	Positive
Early stage CaP	22193M	4+3/10	negative	negative
Early stage CaP	22227M	3+4/10	negative	negative
Early stage CaP	22239P	3+4/10	61 kDa	negative
Early stage CaP	22245B	3+3/10	negative	negative
Early stage CaP	22257W	4+5/10	negative	negative
Early stage CaP	22269K	4+3/10	61 kDa	negative
Early stage CaP	22278S	4+5/10	61 kDa	negative
Early stage CaP	22292G	3+4/10	negative	negative
Early stage CaP	22297C	3+4/10	61 kDa	negative
Early stage CaP	22300K	3+3/10	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative
Normal control		n/a	negative	negative

Table 1